Evidence for activator and repressor functions of the response regulator MtrA from *Corynebacterium glutamicum*

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Abstract

Previous analysis of a *Corynebacterium glutamicum* Δ*mtrAB* mutant showed that the MtrAB two-component signal transduction system influences the expression of genes involved in cell wall metabolism or osmoregulation, but it remained unknown whether this influence is direct or indirect. In order to identify the direct target genes of the response regulator MtrA, chromatin immunoprecipitation as a genome-wide approach and DNA affinity chromatography as a gene-specific approach were performed. The results indicate that *mepA* and *nlpC*, both encoding putative cell wall peptidases, are directly repressed by MtrA, whereas *proP* and *betP*, both encoding carriers for compatible solutes, are directly activated by MtrA.

Introduction

Sensing environmental changes and triggering appropriate responses is often accomplished in bacteria by two-component signal transduction systems [1-3]. The gram-positive soil bacterium *Corynebacterium glutamicum* [4] contains genes for 13 two-component systems [5]. Some of them are highly conserved not only in other *Corynebacterium* species, but also in mycobacteria. One of those is the MtrAB two-component system, which was first described in the human pathogen *M. tuberculosis* [6]. While attempts to disrupt the *mtrA* gene in *M. tuberculosis* H37Rv failed, except when a plasmid-encoded intact *mtrA* copy was present [7], the *mtrAB* genes of *C. glutamicum* could be deleted [8]. The Δ*mtrAB* mutant had a pleiotropic phenotype. In glucose minimal medium, it showed a similar growth rate as the wild type, but entered the stationary phase much earlier due to acidification of the medium. Δ*mtrAB* cells were elongated, segmented and sometimes showed irregular septum formation. The mutant was more sensitive to penicillin, vancomycin and lysozyme, but more resistant to ethambutol. The changes in cell morphology and antibiotics susceptibility indicated that the MtrAB system might be involved in cell wall

metabolism. Using DNA microarrays, dot blots and primer extension studies, genes with an altered mRNA level in the mutant were identified. Some of them showed increased expression in the mutant, such as *mepA* encoding a putative secreted metalloprotease and *ppmA* encoding a putative membrane-bound protease modulator, whereas others display decreased expression such as *betP* encoding a betaine uptake carrier and *proP* encoding a proline uptake carrier. In summary, the analysis of the *mtrAB* mutant indicated that MtrAB is involved in cell wall metabolism and in osmoprotection [8]. In this study we analysed whether increased expression of *mepA* or *ppmA* is responsible for the phenotype of the Δ*mtrAB* mutant and searched for direct target genes of MtrA by chromatin immunoprecipitation and DNA affinity chromatography.

Materials and methods

Bacterial strains, media, and growth conditions

Bacterial strains and plasmids used or constructed in the course of this work are listed in Table 1, oligonucleotides in supplementary Table S1. *C. glutamicum* was routinely cultivated aerobically in 500-ml shaking flasks with 60 ml medium on a rotary shaker (150 rpm) at 30 °C. Either Luria-Bertani (LB) medium [9], CGXII minimal medium [10] or MM1 minimal medium [11] was used. CGXII and MM1 medium contained 2 % or 4 % (w/v) glucose as carbon and energy source. For strain construction and maintenance, BHIS agar plates (BHI agar (Difco, Detroit, USA) with 0.5 M sorbitol) were used. *E. coli* DH5α was grown aerobically on a rotary shaker (150 rpm) at 37 °C in LB medium or on LB agar plates (LB medium with 1.5 % (w/v) agar). If appropriate, kanamycin was added to final concentrations of 25 µg/ml (*C. glutamicum*) or 50 µg/ml (*E. coli*). For DNA affinity chromatography cells were grown in shaking flasks using CGXII medium with 4 % (w/v) glucose and harvested in the exponential growth phase at an OD_{600} of 5 to 6.

General DNA techniques and sequence analyses

Standard methods like PCR, restriction or ligation were carried out according to Sambrook *et al*. [9]. *E. coli* was transformed according to Inoue *et al.* [12]. DNA sequencing was performed with a 3100-Avant genetic analyzer (Applied Biosystems, Darmstadt, Germany). Sequencing reactions were carried out with the ABI PRISM™ Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems).

Mutant and plasmid construction

Deletion mutants of *C. glutamicum* were constructed via a two-step homologous recombination procedure as described previously [13]. The primers used for this purpose are listed in Table S1. The chromosomal deletions were confirmed by PCR with oligonucleotides annealing outside the regions used for deletion (see Table S1). The Δ*mtrA*- and Δ*mtrB*-mutants were also confirmed by Southern blot analysis (for details see [13]) using NcoI-digested (Δ*mtrA*) or NarI-digested (Δ*mtrB*) chromosomal DNA and the corresponding digoxigenin-labelled crossover-PCR product as probe.

Antibiotics susceptibility tests

Quantitative determination of antibiotics susceptibility was performed with Etest® stripes (AB Biodisk, Solna, Sweden) as described previously [8].

ChIP-to-chip analysis

Cultures of the wild type and the Δ*mtrA* mutant were first cultivated overnight in CGXII minimal medium containing 4 % (w/v) glucose. Cells from these precultures were washed in 0.9 % (w/v) NaCl solution and used for inoculation of CGXII minimal medium containing 2 % (w/v) glucose.

At an OD_{600} of 5 – 6, cells from 300 ml culture were harvested by centrifugation (10 min, 11,325 *g*, 4 °C) and washed in 50 ml CGXII medium without MOPS. Subsequently, the cells were resuspended in 10 ml MOPS-free CGXII medium that was supplemented with 1 % (v/v) formaldehyde. After incubation for 20 min at room temperature, glycine was added to a final concentration of 125 mM and the cultures were incubated for another five minutes. Then, cells were harvested and washed twice with 50 ml buffer A (100 mM Tris-HCl, 1 mM EDTA, pH 8.0), resuspended in 10 ml buffer A containing 1 mM phenylmethylsulfonyl fluoride, 1 mM diisopropyl fluorophosphate and 5 mg RNase A and disrupted by five passages at 172 MPa through a French pressure cell (SLM Aminco®, Rochester, USA). The chromosomal DNA of the lysate was sheared by sonication (2 x 30 s with a Branson sonifier 250 using a puls length of 40 % and an intensity of 1) to give an average fragment size of 200 to 1,500 bp. Cell debris was removed by centrifugation at 5,300 *g* for 20 min at 4 °C and the supernatant was used for immunoprecipitation. MtrA protein and MtrA-DNA complexes in the supernatant were immunoprecipitated for 2 h at 4 °C using 400 µl affinity-purified polyclonal rabbit antibodies raised against purified histidine-tagged MtrA protein followed by incubation with 800 µl of 50 % protein A-agarose slurry (Pierce, Rockford, USA.) for one hour at 4 °C. The slurry was washed two times with 10 ml buffer B (50 mM HEPES pH 7.5, 150 mM NaCl, 1 % (v/v) Triton X-100, 0.1 % (w/v) sodium deoxycholate), two times with 10 ml buffer C (buffer B with 400 mM NaCl), two times with 10 ml buffer D (10 mM Tris-HCl pH 8.0, 250 mM LiCl, 0.5 % (v/v) NP-40, 0.5 % sodium deoxycholate, 1 mM EDTA) and two times with 10 ml buffer A. The slurry was resuspended two times in 500 µl elution buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA, 1 % (w/v) SDS), incubated for 10 min at 65 °C and centrifuged. The two supernatants were incubated overnight at 65 °C to reverse formaldehyde-induced crosslinks and then treated for 3 h at 55 °C with proteinase K (400 µg/ml). The DNA was purified by phenol-chloroform extraction,

precipitated with ethanol, washed with 70 % (v/v) ethanol, dried and resuspended in 50 µl H_2O. The DNA of both elution steps was combined. PCR amplification of the co-immunoprecipitated DNA was done according to protocols A and B described at http://derisilab.ucsf.edu/pdfs/Round_A_B_C.pdf. Fluorescence labelling was done as described at http://derisilab.ucsf.edu/pdfs/GenomicDNALabel_A.pdf. Hybridisation to the *C. glutamicum* whole-genome microarray and array scanning were performed as described [8].

DNA affinity chromatography and immunoblotting

Enrichment of DNA-binding proteins interacting with the upstream regions of *mepA, nlpC*, *betP*, *proP*, *NCgl0102-0103*, *nrdH-nadE*, *icd*, *odhA*, *sucCD* and *sdhCAB* was performed as described before [14, 15]. The corresponding regions were amplified using the oligonucleotides mepA_promoter_fw/mepA promoter_rv, nlpC_promoter_fw/nlpC promoter_rv, etc. (see Table S1). Proteins binding unspecifically were washed off with a buffer containing 0.1 M NaCl and specifically bound proteins were subsequently eluted with a buffer containing 2 M NaCl. The proteins present in the high-salt eluate were separated on 15% SDS polyacrylamide gels and electroblotted onto nitrocellulose membranes (Hybond-C extra, Amersham). The blots were incubated with affinity-purified rabbit anti-MtrA antibodies in a 1:10,000 dilution and subsequently with anti-rabbit-IgG-alkaline phosphatase conjugate in a 1:3,000 dilution. Immunoreactive proteins were detected by chemiluminescence using the CDP-Star reagent according to the instructions of the manufacturer (Roche Diagnostics, Mannheim, Germany).

Results and discussion

Phenotypic characterisation of mutant strains

Analysis of global gene expression of the *C. glutamicum* Δ*mtrAB* mutant had indicated that three genes showed a more than threefold increased mRNA level compared to the wild type: *mepA* encoding a secreted metallopeptidase, *ppmA* encoding a putative membrane-bound protease modulator and *lpqB* encoding a lipoprotein of unknown function [8]. The *lpqB* gene is located immediately downstream of *mtrB* and presumably co-transcribed [8]. If increased expression of one of these genes is responsible for the altered cell morphology and antibiotics susceptibility of the Δ*mtrAB* mutant, a deletion of that gene might abolish the phenotype. Therefore, the mutant strains Δ*mtrAB*Δ*mepA* and Δ*mtrAB*Δ*ppmA* were constructed. All attempts to obtain the deletion mutant Δ*mtrAB-lpqB* were not successfull. We tried both a deletion of the *lpqB* gene in the Δ*mtrAB* background and a deletion of the entire *mtrAB-lpqB* gene cluster in the wild-type background. The failure to delete *lpqB* might be a hint at an essential function of this gene. Consistent with the fact that a *mtrAB* deletion mutant could be obtained, it was also possible to delete each of these genes separately, resulting in strains Δ*mtrA* and Δ*mtrB*.

The triple mutants Δ*mtrAB*Δ*mepA* (Fig. 1A) and Δ*mtrAB*Δ*ppmA* (data not shown) exhibited the same growth behaviour in CGXII minimal medium with 4% (w/v) glucose as the Δ*mtrAB* mutant, characterized by a wildtype-like growth rate in the exponential phase and an early growth arrest due to a strong acidification of the medium. The cell length was slightly reduced compared to the Δ*mtrAB* mutant and the susceptibility towards penicillin, vancomycin and ethambutol was very similar to that strain Δ*mtrAB* (Table 2). Thus, increased expression of either *mepA* alone or of *ppmA* alone is not responsible for the pleiotropic phenotype of the Δ*mtrAB* mutant. In contrast to the triple mutants, the Δ*mtrA* and Δ*mtrB* single mutants differed in some aspects from the Δ*mtrAB* mutant. Growth of the Δ*mtrA* strain in CGXII medium with 4% glucose (Fig. 1A) did not lead to an acidification of the medium (Fig. 1B) and glucose was consumed completely. After

entering stationary phase, a decrease in the optical density of the Δ*mtrA* culture occurred, indicating a partial lysis of the cells. A similar behaviour was observed for the Δ*mtrB* strain (data not shown). The cell length of Δ*mtrA* and Δ*mtrB* cells was increased compared to wild-type cells, but much shorter than that of Δ*mtrAB* cells (Table 2). With respect to antibiotics susceptibility, the Δ*mtrA* and Δ*mtrB* strains resembled the Δ*mtrAB* mutant with one exception: the ethambutol sensitivity of the Δ*mtrB* mutant was similar to that of the wild type and much lower than that of the other mutants (Table 2).

Search for MtrA targets using ChIP-to-chip analysis

The transcriptome comparison of Δ*mtrAB* mutant and wild type reported previously [8] allowed the identification of genes whose expression is altered as a consequence of the *mtrAB* deletion, but not the distinction whether these genes are directly or indirectly controlled by MtrA. In order to identify direct target genes of the response regulator MtrA on a genome-wide scale, chromatin immunoprecipitation was combined with DNA chip technology (ChIP-to-chip method). The procedure started with *in vivo* crosslinking of MtrA to its target DNA by treating intact cells with formaldehyde and was followed by cell disruption, DNA shearing by sonification and immunoprecipitation of the MtrA-DNA complexes with anti-MtrA antibodies. After reversal of the crosslinks, the co-precipitated DNA was purified, amplified and labelled with fluorescent nucleotides and then hybridized with a whole-genome DNA microarray in order to identify the DNA fragments [16, 17]. Here, DNA immunoprecipitated from the wild type was compared to DNA immunoprecipitated from the Δ*mtrA* mutant. The experiment was performed three times starting from independent cultures and the results were averaged. The enrichment factor for a given gene or genome region was calculated as fluorescence ratio of immunoprecipitated DNA from the wild type *versus* immunoprecipitated DNA of the Δ*mtrA* mutant. In general, the

enrichment factors observed in our experiments were quite low and only three gene regions were enriched more than two-fold, namely the promoter region of *nlpC* (*NCgl2108*, up to 3.2-fold enrichment), the region covering *NCgl0102* and *NCgl0103* (up to 2.3-fold enrichment), and the region between the divergently organized genes *nrdH* (*NCgl2445*) and *nadE* (*NCgl2446*) (up to 3.7-fold enrichment). The *nlpC* gene encodes a secreted protein with an N-terminal signal peptide (probable cleavage site behind alanine-35) and a carboxyterminal domain belonging to the NlpC/P60 superfamily [18] which might function as cell wall peptidase. The genes *NCgl0102* and *NCgl0103* encode small membrane proteins of unknown function, *nrdH* encodes a glutaredoxin-like protein that could be involved in electron transport for ribonucleotide reductase (the corresponding genes are located nearby), and *nadE* encodes an NAD^+ synthetase. Remarkably, only the *nlpC* gene displayed a more than 2-fold altered (increased) mRNA level in the transcriptome comparison of Δ*mtrAB* mutant *vs.* wild type (see supplementary data to [8]), while the genes located next to the other two enriched regions (*NCgl0102-NCgl0103* and *nrdH-nadE*) did not show significantly altered mRNA levels in the microarray experiments. Therefore, the relevance of MtrA for the expression of these genes is not yet clear. Most of the genes showing altered mRNA ratios in the previous microarray studies showed enrichment factors between 1.0 and 1.5 in the ChIP-to-chip experiments, e.g. *mepA* (1.5), *proP* (1.0), or *betP* (1.1), not allowing a clear decision on whether they are direct target genes of MtrA. Eventually, the procedure used during the initial steps of the ChIP-to-chip experiment has influenced the phosphorylation state of MtrA and thereby altered its binding affinity for different target promoters.

DNA affinity chromatography with selected promoter regions

As an alternative approach to answer the question whether *mepA*, *proP* and *betP* are direct target genes of MtrA, DNA affinity chromatography was performed with the promoter region of these genes and *C. glutamicum* cell extracts. Furthermore, the promoter regions of *nlpC*, *nrdH-nadE* and *NCgl0102-NCgl0103* enriched in the ChIP-to-chip experiments were tested and, as negative controls, the promoter regions of *icd* (isocitrate dehydrogenase), *odhA* (2-oxoglutarate dehydrogenase, E1 subunit), *sucCD* (succinyl-CoA synthetase) and *sdhCAB* (succinate dehydrogenase). The proteins specifically enriched with the promoter regions were tested for the presence of MtrA by Western blot analysis. MtrA was clearly identified in the protein eluates with the promoter fragments of *mepA*, *proP*, *betP*, *nlpC* and *nrdH-nadE* (Fig. 3). In the high-salt eluates obtained with the promoter regions of *NCgl0102-NCgl0103* and the negative controls *icd*, *odhA*, *sucCD* and *sdhCAB*, MtrA was not detectable by western blot analysis (Fig. 3).

Target genes of MtrA in *C. glutamicum, M. avium* and *M. tuberculosis*

Combining the results of the transcriptome comparisons of Δ*mtrAB* mutant and wild type [8], of the ChIP-to-chip experiments and of the DNA affinity chromatography studies, at least four genes are presumably direct target genes of MtrA in *C. glutamicum*: *mepA*, *nlpC*, *betP* and *proP*. As the mRNA levels of *mepA* and *nlpC*, both encoding putative cell wall peptidases, were increased in the Δ*mtrAB* mutant, MtrA functions as a repressor of these genes. On the other hand, the mRNA levels of *betP* and *proP*, encoding carriers for compatible solutes, were decreased in the Δ*mtrAB* mutant, indicating that MtrA functions as an activator of these genes. Thus it appears that MtrA can function both as an activator and as a repressor. In the case of the promoter region of the divergent genes *nrdH* and *nadE* both the ChIP-to-chip assay and DNA affinity chromatography showed binding of MtrA. However, as the mRNA levels of these two genes

were not significantly altered in the microarray experiments [8], further studies are necessary to show an influence of MtrA on the expression of at least one of these genes.

Recently, an *mtrB* mutant of *Mycobacterium avium* was described. It was more sensitive to penicillin, ciprofloxacin (gyrase inhibitor) and clarithromycin (inhibits protein biosynthesis), showed an increased permeability to the cell-permeating green fluorescent nucleic acid stain SYTO16, and exhibited an elongated cell shape [19]. Some of these properties resemble those of the *C. glutamicum* mutants described above. The *M. avium mtrB* mutant was unable to survive intracellularly within THP1 cells and showed decreased expression of a number of genes encoding Mce proteins (mce stands for "mammalian cell entry"), which are located on the outer surface of mycobacterial cells and have been associated with virulence. Whether these *mce* genes are directly controlled by the MtrAB system is unknown. *C. glutamicum*, which is a nonpathogenic species, does not possess orthologs of the *mce* genes.

As indicated before, the *mtrA* gene of *M. tuberculosis* was reported to be essential [7]. Evidence was recently provided that in an MtrA-overproducing strain of *M. tuberculosis* the essential replication gene *dnaA* could be a direct target of MtrA, since its expression was strongly induced [20]. This and several other effects of *mtrA* overexpression where not observed, however, when both *mtrA* and *mtrB* were overexpressed. In our transcriptome studies with the Δ*mtrAB* mutant of *C. glutamicum*, no changes in the mRNA level of *dnaA* (*NCgl0001*) compared to the wild type were observed [8]. Similarly, the promoter region of *dnaA* was not enriched in the ChIP-to-chip experiments. We cannot exclude of course that *dnaA* expression is induced in *C. glutamicum* upon overexpression of *mtrA*.

The studies performed up to now on the function of MtrAB in *C. glutamicum*, *M. avium* and *M. tuberculosis* indicate that this two-component system influences the expression of different genes in the different species. However, due to the high similarity in terms of sequence and

genomic organisation of MtrAB, it would be surprising if there are no common target genes and the phenotype of the *M. avium mtrB* mutant supports this view.

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Table 1

Strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Source or reference
Strains		
E. coli DH5α	F^- ϕ80*dlac*Δ(*lacZ*)M15 Δ(*lacZYA-argF*) U169 *endA1 recA1 hsdR17* (r_K^- , m_K^+) *deoR thi-1 phoA supE44* λ^- *gyrA96 relA1*	Invitrogen (Karlsruhe, Germany)
C. glutamicum ATCC13032	Biotin-auxotrophic wild-type strain	[21]
C. glutamicum Δ*mtrAB*	Derivative of ATCC13032 with an in-frame deletion of the *mtrAB* genes	[8]
C. glutamicum Δ*mtrAB*Δ*mepA*	Derivative of the Δ*mtrAB* strain with an additional in-frame deletion of the *mepA* gene	This work
C. glutamicum Δ*mtrAB*Δ*ppmA*	Derivative of the Δ*mtrAB* strain with an additional in-frame deletion of the *ppmA* gene	This work
C. glutamicum Δ*mtrA*	Derivative of ATCC13032 with an in-frame deletion of the *mtrA* gene	This work
C. glutamicum Δ*mtrB*	Derivative of ATCC13032 with an in-frame deletion of the *mtrB* gene	This work
Plasmids		
pK19*mobsacB*	Km^R; mobilisable *E. coli* vector used for allelic exchange in *C. glutamicum* (pK18 $oriV_{E.c.}$, *sacB*, *lacZ*α)	[22]

pK19Δ*mepA*	Km^R; pK19*mobsacB* derivative containing a crossover PCR product fusing the upstream and the downstream region of *mepA*	This work
pK19Δ*ppmA*	Km^R; pK19*mobsacB* derivative containing a crossover PCR product fusing the upstream and the downstream region of *ppmA*	This work
pK19Δ*lpqB*	Km^R; pK19*mobsacB* derivative containing a crossover PCR product fusing the upstream region of *lpqB* (in the Δ*mtrAB* mutant) and the downstream region of *lpqB*	This work
pK19Δ*mtrAB lpqB*	Km^R; pK19*mobsacB* derivative containing a crossover PCR product fusing the upstream region of *mtrA* and the downstream region of *lpqB*	This work
pK19Δ*mtrA*	Km^R; pK19*mobsacB* derivative containing a crossover PCR product fusing the upstream and the downstream region of *mtrA*	This work
pK19Δ*mtrB*	Km^R; pK19*mobsacB* derivative containing a crossover PCR product fusing the upstream and the downstream region of *mtrB*	This work

Table 2

Phenotypic properties of *C. glutamicum* mutant strains analysed in this work.

Strain	Growth rate μ[a] (h^{-1})	Maximal OD_{600}[a]	Cell length[b] (μm)	MIC[c] [μg/ml]		
				Penicillin	Vancomycin	Ethambutol
13032	0.40 ± 0.03	59 ± 4	1.5 ± 0.2	0.45 ± 0.07	0.92 ± 0.14	1.7 ± 0.27
Δ*mtrAB*	0.42 ± 0.05	31 ± 4	3.7 ± 0.3	0.15 ± 0.07	0.45 ± 0.19	>256
Δ*mtrAB*Δ*mepA*	0.40 ± 0.01	34 ± 8	2.9 ± 0.8	0.13 ± 0.08	0.55 ± 0.26	>256
Δ*mtrAB*Δ*ppmA*	0.38 ± 0.02	33 ± 8	2.8 ± 0.4	0.17 ± 0.09	0.47 ± 0.06	>256
Δ*mtrA*	0.45 ± 0.06	43 ± 6	2.1 ± 0.6	0.20 ± 0.16	0.53 ± 0.14	>256
Δ*mtrB*	0.44 ± 0.02	40 ± 6	1.8 ± 0.4	0.26 ± 0.07	0.50 ± 0.00	4.33 ± 1.53

[a] Growth rate and maximal OD_{600} were determined for at least 3 independent cultivations in CGXII minimal medium with 4% (w/v) glucose.

[b] Cell length was determined for cells cultivated in MMI medium.

[c] Minimal inhibitory concentrations (MICs) were determined with Etest® stripes as described previously [8]. Mean values and standard deviations were determined from at least three independent experiments.

Table 3

PCR fragments used for DNA affinity chromatography

Gene	Position of PCR fragment with respect to the translation start site	Length of PCR fragment
mepA	-511 to +59	570 bp
nlpC	-496 to +52	548 bp
betP	-378 to +54	432 bp
proP	-506 to +59	565 bp
NCgl0102	-155 to +420	575 bp
NCgl2445	-500 to +50	550 bp

Figure legends

Fig. 1. Growth (A) and pH changes (B) of *C. glutamicum* wild type (filled circles) and the mutants Δ*mtrAB*Δ*mepA* (open circles) and Δ*mtrA* (filled triangle) in CGXII minimal medium with 4 % glucose as sole carbon source.

Fig. 2. Cell morphology of *C. glutamicum* wild type (A) and the mutants Δ*mtrA* (B), Δ*mtrB* (C), Δ*mtrAB* (D), Δ*mtrAB*Δ*mepA* (E) and Δ*mtrAB*Δ*ppmA* (F) after overnight growth in MMI minimal medium with 2 % glucose. Photographs were taken at a 1000-fold magnification using a Leica microscope DM-LB (Leica Microsystems, Wetzlar, Germany) equipped with CallCam video camera (Phase, Lübeck, Germany).

Fig. 3. Western blot analysis with affinity-purified anti-MtrA antibodies and the high-salt eluates obtained by DNA affinity chromatography with the promoter regions of *nlpC*, *betP*, *mepA*, *proP*, *nrdH-nadE*, *NCgl0102-0103*, *icd*, *odhA*, *sucCD* and *sdhCAB*.